Express Mail Label No. EL 405 594 443 US

06-14-00 A

06/13/00

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 0152.00372
Total Pages in this Submission

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

jc511 U.S. PTO 09/593629 06/13/00

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

SERTOLI CELLS AS BIOCHAMBERS

and invented by:

Don F. Cameron; Paul R. Sanberg; Samuel Saporta; Joel J. Hushen

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having **28** pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☐ Cross References to Related Applications *(if applicable)*
   - c. ☒ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

"EXPRESS MAIL" Mailing Label Number EL405594443US

Date of Deposit 6-13-00

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office To Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Constance McLear

(Signature of person mailing paper or fee)

P01USML/REV03

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 0152.00372
Total Pages in this Submission

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

   a. ☐ Formal b. ☒ Informal Number of Sheets 6

4. ☒ Oath or Declaration

   a. ☐ Newly executed *(original or copy)* ☒ Unexecuted

   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*

   c. ☒ With Power of Attorney ☐ Without Power of Attorney

   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche

7. ☐ Genetic Sequence Submission *(if applicable, all must be included)*

   a. ☐ Paper Copy

   b. ☐ Computer Readable Copy

   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & documents)*

9. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

    ☐ First Class ☒ Express Mail *(Specify Label No.):* EL 405 594 443 US

UTILITY PATENT APPLICATION TRANSMITTAL (Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 0152.00372
Total Pages in this Submission

Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☒ Small Entity Statement(s) - Specify Number of Statements Submitted: 1

17. ☐ Additional Enclosures *(please identify below):*

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	24	- 20 =	4	x $9.00	$36.00
Indep. Claims	7	- 3 =	4	x $39.00	$156.00
Multiple Dependent Claims (check if applicable) ☐					$0.00
				BASIC FEE	$345.00
OTHER FEE *(specify purpose)*					$0.00
				TOTAL FILING FEE	$537.00

☒ A check in the amount of $537.00 to cover the filing fee is enclosed.

☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 11-1449 as described below. A duplicate copy of this sheet is enclosed.

- ☐ Charge the amount of as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: June 13, 2000

Signature

Amy E. Rinaldo, Reg. No. 45,791
KOHN & ASSOCIATES
30500 Northwestern Highway, Suite 410
Farmington Hills, Michigan 48334
(248) 539-5050
FAX: (248) 539-5055

cc:

P01USML/REV03

PATENT

Attorney's Docket Number: 0152.00372

Applicant or Patentee: Cameron et al.

Serial or Patent No:

Filed or Issued:

Title: SERTOLI CELLS AS BIOCHAMBERS

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
[37 CFR 1.9(f) and 1.27(c)]-SMALL BUSINESS CONCERN

I hereby declare that I am:

_____ the owner of the small business concern identified below:

__X__ an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Concern: University of South Florida Research Foundation, Inc.

Address of Concern: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement: (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when, either directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

__X__ the specification filed herewith with title as listed above.

_____ the application identified above.

_____ the patent identified above.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c), if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*** NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)**

Each such person, concern or organization having any rights in the invention is listed below:

_____ No such person, concern, or organization exists.

__X__ Each such person, concern or organization is listed below.

NAME: University of South Florida

ADDRESS: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

___ Individual ___ Small Business _x_ Nonprofit Organization

NAME: _____

ADDRESS: _____

_____

___ Individual ___ Small Business ___ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Kenneth G. Preston

Title in Organization: Executive Director

Address of Person Signing: 4202 East Fowler Avenue - FAC 126

Tampa, Florida 33620-4962

SIGNATURE: _____ **Date:** 6/1/00

Attorney's Docket Number: 0152.00372

Applicant or Patentee: Cameron et al.

Serial or Patent No:

Filed or Issued:

Title: SERTOLI CELLS AS BIOCHAMBERS

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)--NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: University of South Florida

Organization Address: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

Type of Organization:

X University or other Institution of Higher Education

___ Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)

___ Nonprofit Scientific or Educational under Statute of State of the United States of America State: _____
Citation of Statute: _____

___ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3) if located in the United States of America State: _____
Citation of Statute: _____

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and 41(b) of Title 35, of United States Code with regard to the invention described in:

X the specification filed herewith with title listed above.

___ the application identified above.

___ the patent identified above.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or nonprofit organization under 37 CFR 1.9(e).

* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME: University of South Florida Research Foundation, Inc.

ADDRESS: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

___Individual _X_Small Business ___Nonprofit Organization

NAME: _____

ADDRESS: _____

_____

___Individual ___Small Business ___Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: George R. Newkome, Ph.D.

Title in Organization: Vice President for Research

Address of Person Signing: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

SIGNATURE: _____ Date. 6/1/00

Attorney Docket No. 0152.00372

SERTOLI CELLS AS BIOCHAMBERS

GRANT INFORMATION

Supported in part by NASA Grant NAG8-1381.

BACKGROUND OF THE INVENTION

1. TECHNICAL FIELD

The present invention relates to methods of transplanting cells. More specifically, the present invention relates to methods of transplanting cells to create a localized immunosuppressive effect in the tissue receiving the transplanted cells.

2. BACKGROUND ART

The central nervous system (CNS) has poor regenerative capacity which is exemplified in a number of neurodegenerative disorders. An example of such a disorder is Parkinson's disease. The preferred pharmacotherapy for Parkinson's disease is the administration of L-dopa which slows the progression of this disease in some humans. However, the neuropathological damage and the consequent behavioral deficits is not reversed by this treatment protocol.

Laboratory and clinical studies have shown that the transplantation of cells into the CNS is a potentially significant alternative therapeutic modality for neurodegenerative disorders such as Parkinson's disease (Wictorin et al., 1990; Lindvall et al., 1990; Sanberg et al., 1994; Bjorlund and Stenevi, 1985; Freeman et al., 1994). In some cases, transplanted neural tissue can survive

and form connections with the CNS of the recipient (i.e. the host). When successfully accepted by the host, the transplanted tissue (i.e. the graft) has been shown to ameliorate the behavioral deficits associated with the disorder (Wictorin et al., 1990). The obligatory step for the success of this kind of treatment is the prevention of graft rejection (i.e. graft acceptance).

Currently, fetal neural tissue is the primary graft source for neural transplantation (Lindvall et al., 1990; Bjorklund, 1992; Isacson et al., 1986; Sanberg et al., 1994). Other viable graft sources include adrenal chromaffin cells and various cell types that secrete nerve growth factors and trophic factors. The field of neural tissue transplantation as a productive treatment protocol for neurodegenerative disorders has received much attention resulting in its progression to clinical trials. Preliminary results and clinical observations are promising although the graft rejection phenomenon remains problematic.

Transplantation is also a valuable therapy for other diseases, such as insulin dependent diabetes mellitus. Insulin dependent diabetes mellitus is a major health problem. Current forms of therapy are not efficient and do not necessarily lead to a prevention of diabetic complications such as renal failure or blindness. A desirable treatment alternative is to provide the diabetic with an endogenous source of insulin, transplanting either the whole pancreas or the endocrine component of the pancreas (i.e. islets of Langerhans) into the diabetic recipient. Although, whole pancreas transplantation is successfully achieved with at least 60% of the grafts still functioning after transplantation for one year, a major weakness of this approach is the need for continuous immunosuppression with powerful and toxic immunosuppressant drugs.

The transplantation of the isolated islets containing the insulin secreting β-cells has received much attention in both animal models of diabetes (1-7) and in humans (8-16). However, islet transplantation to a variety of organ sites has met with little success as a viable treatment for diabetes. For example, islet transplantation of major histocompatibility complex (MHC) in the BB/W rat with spontaneous diabetes mellitus of autoimmune etiology results in destroyed islets within a few days by a recurrence of the autoimmune disease (17). Likewise, destruction of grafted cells in the diabetic BB/W rat occurs in grafted islets of MHC-incompatible donors (18, 19). In the course of finding a suitable organ or tissue site for islet transplantation, it was discovered that the relocated abdominal testis, in particular, provides an extraordinary safe environment for extended survival of islet grafts and some relief of the diabetic complications (20-22).

The testis has long been considered to be an immunologically privileged site (23-26) although the precise mechanism(s) by which it protects (suppresses) graft rejection has not been clearly defined. Isolated islets of MHC-compatible donors have been shown to survive for extended periods of time in the non-immunosuppressed BB/W rat if implanted in the rat's testis which is then placed into the host's abdominal cavity (20-22,27). Although the maintenance of functional islets allografts is significant, a more difficult task and far more potentially significant accomplishment, in terms of clinical applicability, is the induction of normoglycemia in diabetic animals by the implantation of cross-species islet xenografts.

Selawry and co-workers demonstrated the feasibility of such a procedure by successfully implanting incubated hamster islets into the BB/W rat abdominal testes (22,27,28). As a result of the abdominal testis/islet implant, the diabetic animals in these studies became normoglycemic. Long-

term survival of the islet xenografts did not require prolonged immunosuppression to prevent rejection and to maintain normal sugar levels. In all cases implant viability required the protective milieu of the abdominal testis. It now appears that the donor origin of these isolated islets does not seem to influence their long-term survival. Islet cells grafted against major histocompatibility barriers (21), islet xenografts (27) and islets of MHC-compatible donors grafted into the testes of the diabetic BB/W rats functioned indefinitely in the recipient rendering the once diabetic animal normoglycemic.

The major weakness of this type of islet transplantation protocol is associated with the use of such an unconventional organ site. One major concern is the possibility of malignant transformation of germ cells at the higher core body temperature (29). More importantly, it would not be possible to use this transplantation protocol for the treatment of female diabetics.

Histological examination of grafted abdominal testes has shown that the islet implants are always found within the interstitial compartment of the gonad, which consists of the endocrine cells of Leydig, macrophages, blood vessels, testicular interstitial fluid and extracellular macromolecules (31). Any of the secretory products of these cells are potentially capable of inhibiting the immune response. For instance, Born and Wekerle (32, 33) showed that active suppression of immune responses occurred by Leydig cells *in vitro*. These investigators speculated that the Leydig cells might prevent lymphocyte proliferative responses by creating an "immunologically neutral zone" around the seminiferous tubules and thus decreasing the danger of T-cell infiltration in to the intratubular spaces. It was shown by Williams (34) that leukemic cells accumulate in the interstitial compartment where they are apparently protected against destruction by the host's immune defenses.

The "zone of protection" theory of Born and Wekerle (32) is attractive but it is not likely that this major component of the testicular interstitium, i.e. Leydig cells, is responsible for the synthesis of some protective (immunosuppressant) factor. Treatment of rats with ethane dimethanesulphonate (EDS), which selectively destroys the Leydig cell completely, including steroidogenesis and all other functions, had no adverse effects on the survival of intratesticular islet allografts (30). It is not probable that germ cells were involved either, since these cells are readily depleted in the abdominal testis. By eliminating these cells, Cameron and Sewlary concluded that the Sertoli cell was the most probable testicular cell type providing the testis with its unique immunologically privileged environment and that this cell was most likely responsible for the unexplained absence of islet rejection in abdominal testes (30). Based on these findings, Selawry and Cameron (35) attempted to create a similar immunologically privileged site outside of the testis utilizing Sertoli cells as an immunosuppressant agent. To this end, isolated Sertoli cells were transplanted with isolated islets under the kidney capsule in female diabetic rats (see Figure 1). Results from this study showed this novel transplantation protocol resulted in normoglycemia and that long-term islet allograft survival was achieved in a traditionally immunologically hostile site. We concluded that the Sertoli cell, independent of the testicular milieu, secreted an immunosuppressant factor(s) which was neither androgenic nor inhibitory to ovulation since 6 of the 7 mated recipients became pregnant, carried a pregnancy to term and nursed the pups successfully (35).

For the long-term treatment of diabetes, it is clear that the presence of viable Sertoli cells is a prerequisite for long-term islet graph success and maintenance of long-term beta cell function. We do not yet clearly understand, however, the mechanism(s) which yield this observation. The likely explanation is that the Sertoli cells secret an immunosuppressant

factor(s) which cooperates with exogenous immunosuppressants such as cyclosporine A to prevent a complete immune response and subsequently tissue rejection (35). Sertoli cells are active secretory cell types synthesizing many proteins, some of which promote growth and others which have immunosuppressive capabilities (36, 55). Initial studies to verify such a factor have been positive to date. The effects of Sertoli cell conditioned media on Con A-stimulated spleen lymphocyte proliferation showed that products secreted by Sertoli cells inhibit lymphocyte proliferation in a dose-dependent manner. The synthesis was temperature dependent, occurring predominantly at 37°C and hormone dependent, requiring the presence of follicle stimulating hormone (FSH) in the Sertoli cell culture (see Figure 2). We further examined the mechanism of inhibition of lymphocyte proliferation and showed that preconditioned Sertoli cell media inhibited the production of the lymphokine IL-2 in a dose-dependent manner (see Figure 3A). Because the addition of exogenous IL-2 was not able to reverse this inhibition (see Figure 3B), it appears likely that the preconditioned media inhibited both IL-2 production and T-lymphocyte responsiveness to IL-2 (38) in concurrence with similar finding by DeCesarts et al. (39) It is widely acknowledged that all proliferating T-cells express IL-2 receptors, while resting cells do not, and that interaction of IL-2 with its receptor is an absolute requirement for the clonal expansion of activated T-cells (40). Because the prevention of IL-2 receptor interaction completely inhibits T-cell proliferation, we propose that both clonal expansion and viability of activated T-cells are suppressed by an immunosuppressive factor secreted by the Sertoli cells (35). In this fashion, the putative Sertoli cell derived immunosuppressant would appear to suppress the rejection by a mechanism similar to the action of cyclosporin A which also suppressed the production of IL-2 (41-44).

Although this hypothesis is appealing and with some research support of an indirect nature, it remains to be clearly unravelled. Recently, an additional and even more ppealing hypothesis has received consideration attention. Bellgrau et al. (45*) in a letter to Nature showed that testis grafts that expressed Fas (CD95) ligand (FasL) survived indefinitely when transplanted under the kidney capsule, whereas testis grafts from *gld* mice (FasL deficient) were rejected when transplanted at the same site (45). A reverse transcriptase-polymerase chain reaction analysis demonstrated that Sertoli cells constitutively express FasL mRNA. Additionally, they showed that isolated Sertoli cells derived from normal, but not the *gld* mice survived indefinitely when transplanted under the kidney capsule. They concluded that the expression of functional FasL by Sertoli cells accounts for the immune-privilege nature of testis and suggested a mechanism by which Sertoli cells induce localized immune privilege to islets co-transplanted with Sertoli cells in an otherwise immune hostile site (i.e. subjacent the kidney capsule). They pointed out that FasL ligand-mediated immunosuppression would be expected to primarily target activated effector T cells rather than the activation steps that produce them, a mechanism by which Cyclosporin A produces immunosuppression. This would suggest that by targeting only activated T lymphocytes, grafted cell-associated FasL may provide a highly specific form of immunosuppression for ameliorating T-cell-dependent graft rejection. To this end, Lau et al, (46) transfected muscles cells with the FasL gene and co-transplanted them with islets beneath the kidney capsule and achieved local immunoprotection for the grafted islet, albeit for only 80 days. In a letter to Science, D. Green declared this a stunning advance and declared that "It's almost the Holy Grail of immunosuppression to restrict the suppression to the environment of the graft" (47). Selawry and Cameron (35) achieved the same results with long-term immunoprotection of the grafted islets and long-term maintenance of normoglycemia in the diabetic rat by co-transplanting the islets with the natural producer of FasL, Sertoli cells. The salient features of

terminally differentiated Sertoli cells that make them important and preferable as a transplantation facilitator are 1) they live for the life of the donor and may survive for the life of the recipient host (providing, thereby, long-term FAS-L induced local immunoprotection for the transplanted tissue or cells), 2) they do not divide and 3) they are easily isolated.

Since Sertoli cells secrete many growth enhancing factors including insulin-like growth factor I (55), the presence of Sertoli cells, in addition to their immunoprotective protective properties, may provide additional tropic and growth support to the transplant. Recently, Selawry et al, (48) showed that when cryopreserved pig Sertoli cells were thawed and immediately place in culture with Sertoli cells, there was a significant enhancement of post-thaw survival and insulin secretion when compared to thawed islets not co-cultured with Sertoli cells. They suggested that insulin-like growth factor I may have provided growth factor support to the cell membrane known to be damaged during freezing. Recently Sanberg et al (49-51) demonstrated that Sertoli cells can survive in the brain and, in fact, protect bovine adrenal chromaffin cell xenografts from rejection when co-transplanted into the striatum of the Parkinson's disease rat model. Even more significant, Sertoli cells alone transplanted into the PD rat result in the amelioration of motion dysfunction to the same degree as do chromaffin cells indicating a type of successful growth factor therapy, as yet unknown, provided for by the transplanted Sertoli cells (52). Similar to islet cells, Cameron et al (53) have shown that the post-thaw viability of fetal brain cells is significantly enhanced if the neuron are co-cultured with Sertoli cells again indicating the generalized ability of Sertoli cell secretory products to support the viability of isolated cells. For both islets and neurons, the growth and viability enhancing characteristics of Sertoli cells were evident only when the Sertoli cells were present as opposed to only media soluble factors found in expended pre-conditioned Sertoli cell media.

The extra-testicular utilization of Sertoli cells in facilitated transplantation holds enormous potential based of the cell's ability to provide for long-term localized immunosuppression and generalized growth enhancement of the transplanted cells and tissues. There is a distinct advantage to utilizing whole Sertoli cells rather than specific growth or immunosuppressant factors in that the Sertoli cell appears to continue expressing its desirable transplantation facilitation properties as long as the cell survives in the host, which may be for the life of the recipient. Because Sertoli cells cease mitotic activity following differentiation (54) and do not appear to re-acquire it following transplantation, it may be possible to transplant a stable population of Sertoli cells which remains stable for the life of the host. It is not an understatement to recognize that the utilization of extra-testicular Sertoli cells as transplantation facilitators opens the window to new and potentially significant protocols for transplantation success and represents the beginning "of a new era in transplantation " therapy (47).

In general, systematic immunosuppression is necessary if successful transplantation is to be achieved in humans. Immunosuppression of the entire body (i.e. systemic) can result, eventually, in graft acceptance. It is acquired, however, by placing the individual at medical risk making the immunosuppressant therapy itself more of a liability than a benefit in some cases. For a lack of a better immnosuppressant treatment, systemic immunosuppressants, with Cyclosporine-A (CsA) as the treatment choice, have been used as adjunctive therapy in neural transplantation protocols (Sanberg et al., 1994; Freeman et al., 1994; Borlongan et al., 1995). Arguably, systemic CsA treatment may be contraproductive to successful graft acceptance in the CNS because of its systemic effect and because CsA itself has been shown to cause detrimental side effects and may in fact, be cytotoxic to neural tissues (Berden et al., 1985; deGroen et al., 1984).

It would be useful to develop a mechanism that enhances the productive cell transplantation techniques already utilized for neurodegenerative disorders, such as Parkinson's disease. This mechanism should improve these protocols in ways which would more effectively slow the neurodegenerative disease process, more actively promote the re-establishment of normal neural tissue physiology and better alleviate the functional disabilities associated with the neural tissue dysfunction. Likewise, it would be useful to provide trophic support for the transplanted cells. Further, it would useful if this support lead to the reduction or elimination of systemic immunosuppression while maintaining the ability to immunosuppress locally (i.e. at the graft site) by an immunosuppressant which is biologically tolerated by the host. Sertoli cells may provide this desired option since it is clear from the diabetic studies, as summarized above, that co-transplantation with Sertoli cells will deliver local immunosuppression and promote, therefore, efficient graft acceptance and functional restoration of the tissue-related dysfunction.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a biological chamber including outer walls of Sertoli cells and an inner lumen. Also provided is a transplantation facilitator including a biochamber which is formed from an engineered Sertoli tissue construct. A method of making biochambers by co-culturing facilitator cells and therapeutic cells is also provided. Additionally, there is provided a method of transplanting cells by incorporating therapeutic cells into a biochamber and transplanting the biochamber containing the therapeutic cells. Further, a method of treatment using these engineered biochambers is also included.

DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a diagram showing the formation of a biochamber on a substrate;

Figure 2 is a comparison showing the differences between the conventional culture and a microgravity co-culture;

Figure 3 is a mechanism showing the way the Sertoli cells effect immunosuppression at the graft site;

Figure 4 is a photograph showing Sertoli cells (SC) and islets (arrows) in a Sertoli-islet tissue construct created in a conventional co-culture; B-cells are immunostained for insulin;

Figure 5 is a photograph of Sertoli cells (SC) and B-cells (arrows) in a Sertoli-islet tissue construct created in a conventional co-culture, B-cells are immunostained for insulin;

Figure 6 is a photograph of Sertoli-Neuron-Aggregate-Cells (SNACS for *in vitro* following coculture of rat Sertoli cells and NT2 neuros in simulated microgravity utilizing the High Aspect Rotation Velocity (HARV) bioreactor; and

Figure 7 is a photograph of Immunocytochemical staining of mouse FasL and human nuclear matrix proteins in Sertoli-Neuron Aggregated Cells (SNACs) following HARV incubated cocultures.

DETAILED DESCRIPTION OF THE INVENTION

Generally, the present invention provides a biological chamber system which is used for transplanting cells. More specifically, the biochamber is formed of facilitator cells such as, but not limited to, Sertoli cells which form a chamber or vessel having an inner cavity or lumen containing therein a population of cells different than the facilitator cells. In the preferred embodiment, this population of cells include therapeutic cells.

By "Biochamber" or "vessel", it is meant that a number of cells are engineered in such a manner as to form discrete walls about a lumen or center chamber. More specifically, the biochamber is formed by a structural modification of the Sertoli cells, this new structure being similar to the original Sertoli cell structure prior to cell harvesting. It is during this harvesting that the Sertoli cells are reorganized to form a central lumen in which the therapeutic cells are housed within a newly formed micro-environment. This micro-environment can contain therein therapeutic cells, which are used for transplantation. By "facilitator cell", as used herein, it is meant to include a cell which is able to provide localized immunosuppression or otherwise facilitate or make more effective the transplant. The facilitator cells provide bio-protection for the therapeutic or transplanted cells. This bio-protection includes, but is not limited to, protection from a biological source such as an immune response, whether cellular or humoral. In the preferred embodiment, the facilitator cell is a Sertoli cell. Such cells, as described hereinbelow, are able to reorganize to form walls defining an inner lumen. The biological/living

walls provide a physical as well as an immunological barrier for the cells contained therein. The apical secretions of Sertoli cells contribute to the unique trophic-bridge micro-environment of the luminal spaces in which therapeutic cells reside.

The term "therapeutic cell" as used herein, is meant to include the cells to be transplanted. For example, these therapeutic cells can include, but are not limited to, the following cells: dopaminergic cells, pancreatic islet cells, bovine chromaffin cells and immortalized neuron-like NT2 cells. The cells are therapeutic in that they can secrete hormones, factors, or the like that can have a therapeutic effect upon the host. They, like the Sertoli cell walls, are biosensitive in that they can respond to factors in their environment.

By modifying the harvested Sertoli cell by the methods of the instant invention that the cells reorganize into a tissue structure similar to that observed in the testis. They become a protective and nurturing barrier tissue, encapsulating the therapeutic cells in a unique micro-environment. Because the engineered Sertoli tissue construct captures the therapeutic cells in their new environment, this produces a dynamic support system for the therapeutic cells whereby the discreet units become efficient and viable within this special structure. Each biochamber becomes a discreet transplant unit, both nurtured and immunoprotected by the surrounding engineered Sertoli tissue.

In the preferred embodiment, Sertoli cells are isolated from a mammal, such as, but not limited to a prepubertal rat or pig testes and co-cultured with a therapeutic cell type in a culture environment that enhances tissue formation. This can be accomplished by co-culturing the different cell types in simulated microgravity culture utilizing the HARV bioreactor or other culture technologies. In a further embodiment, the co-culturing is performed without the microgravity environment.

The addition of a basement membrane-like extracellular matrix to the incubation medium induces the epithelization and polarization of Sertoli cells, and subsequent formation of Sertoli-Sertoli junctional complexes between adjacent Sertoli cells, and the formation of a lumen or lumina. There is segregation of the Sertoli cells away from the therapeutic cells during the process of Sertoli cell epithelization leaving the therapeutic cells residing within the newly-formed luminal spaces. The luminal space(s) is/are created during this reorganization of the Sertoli cells and the formation of the Sertoli-Sertoli junctions. These junctions form an intraepithelial barrier similar to that observed in the testis and referred to as the blood-testis barrier. Apical polarization of Sertoli cell secretion is the likely mechanism by which the lumen is formed (Figures 1-3).. The reorganized Sertoli cells illustrated in Figures 1-3create an item which is referred to as the Sertoli cell biochamber.

The Sertoli cell portion of the biochamber acts as a facilitator or a bridge cell for the transfer of material into and out of the lumen.

Examples of such biochambers, include but are not limited to, Islet-filled Sertoli cell biochambers (SICAs) and NT2 cell-filled biochambers (SNACs) which exemplify how therapeutic cell types can be incorporated into the Sertoli cell biochamber. SICAs secrete insulin in response to a glucose challenge (180 mg %) and also suppress activated lymphocytic proliferation (16). Similarly, SNACs enhance the differentiation of NT2 cells to the dopaminergic phenotype (17.18) and likewise provide for immunoprotection of the neurons as judged by the expression of FasL on the Sertoli cells (see Fig 3). SICAs and SNACs are therapeutic cell-filled Sertoli cell biochamber products created by this tissue engineering protocol and are designed for the use in therapeutic transplantation treatments for serious diseases such as diabetes and Parkinson's disease.

Since Sertoli cells are terminally differentiated, and the cells are mitotically inactive. They live for a long period of time, and potentially as long as any therapeutic cell type that can be engineered into the Sertoli cell biochamber. If transplanted in a Sertoli cell biochamber, therapeutic cells can be protected against immune surveillance and subsequent rejection in a micro-environment (provided for by Sertoli cell secreted growth and trophic factors) that also maintains and stimulates their functional phenotypes on a long-term basis. This has a significant impact on the successful transplantation treatment of many serious diseases and on the status of transplantation biology in general.

The above discussion provides a factual basis for the use of Sertoli cell biochambers. The methods used with and the utility of the present invention can be shown by the following non-limiting examples and accompanying figures.

EXAMPLES

GENERAL METHODS:

General methods in molecular biology: Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989), and in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989) and in Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988), and in Watson et al., *Recombinant DNA*, Scientific American Books, New York and in Birren et al (eds) *Genome Analysis: A Laboratory Manual Series, Vols. 1-4* Cold Spring Harbor Laboratory Press, New York (1998) and methodology as set forth in United

States patents 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057 and incorporated herein by reference. Polymerase chain reaction (PCR) was carried out generally as in *PCR Protocols: A Guide To Methods And Applications*, Academic Press, San Diego, CA (1990). In-situ (In-cell) PCR in combination with Flow Cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni et al, 1996, Blood 87:3822.)

General methods in immunology: Standard methods in immunology known in the art and not specifically described are generally followed as in Stites et al.(eds), Basic and Clinical Immunology (8th Edition), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), Selected Methods in Cellular Immunology, W.H. Freeman and Co., New York (1980).

Immunoassays

In general, immunocytochemistry ELISAs are the preferred immunoassays employed to assess a specimen. These assays are well known to those skilled in the art. Both polyclonal and monoclonal antibodies can be used in the assays. Where appropriate other immunoassays, such as radioimmunoassays (RIA) can be used as are known to those in the art. Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States patents 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521 as well as Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor, New York, 1989

Delivery of gene products/therapeutics (compound):

The compound of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical

condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

The biochambers of the instant invention can be administered in various ways. These include subcutaneously or parentally, including intravenous, intraarterial, intramuscular, intraperitoneal and intranasal administration. Pharmaceutically acceptable carriers, diluents, adjuvants and vehicles are also useful for administration of the biochambers. These refer to any diluent, carrier, adjuvant or vehicle as commonly known to one of ordinary skill in the art.

EXAMPLE 1:

Recently, Sertoli cells have been utilized to facilitate islet transplantation on the basis that the testis-derived cells provide localized immunoprotection at the graft site and stimulate islet viability. The relationship between Sertoli cells and β-cells is not yet well defined *in vivo* nor *in vitro*. To further evaluated this relationship and to promote Sertoli/islet cell 3-dimensional aggregation (SICA) *in vitro*, Sertoli cells and islets were co-cultured in simulated microgravity using the NASA high aspect rotation velocity (HARV) bioreactor.

Sertoli cells, harvested from mammals by methods as known by those

of skill in the art, and islets, obtained by methods known to those of skill in the art as in Korbutt et al, were isolated from neonatal pigs by routine enzymatic digestion. Sertoli cells were placed immediately into HARVs at the time of isolation. Isolated islets were pre-cultured in flasks for 14 days (to expedite the removal of exocrine tissue) prior to incubation in HARV's with or without Sertoli cells. HARV co-cultures were incubated at 37° for 28 days in defined incubation medium consisting of DMEM; F-12 supplemented with ITS+ Retinol, and 1% Matrigel . Every 48 hours, 4 ml of media was removed and replaced with fresh media. The SICAs were exposed to a standard glucose challenge (180 mg% glucose) after which samples were collected every ten minutes for an hour and subsequently assayed for insulin by radioimmunoassay. Cell viability was determined by trypan blue exclusion, the presence of β-cells was determined by differential staining with dipherrylthiocarbazone and/or insulin immunostaining, and Sertoli cells were determined by FAS-L immunostaining.

By the end of the incubation period, Sertoli cells and islets had formed sizable (3-10 mm diameter) tissue constructs, with those formed in Matrigel mediums being larger. Cell viability was high (>80%) and β-cells were detected in both SICAs In the three separate HARV incubations, the presence of Sertoli cells in SICA's enhanced the basal and total amount of insulin secreted in response to the glucose challenge when compared to islet-only HARV monocultures. In the presence of Sertoli cells, the SICA's insulin response to the elevated glucose was quicker and appeared to be prolonged.

EXAMPLE 2:

FORMATION OF SERTOLI-NEURON AGGREGATED CELLS(SNACs) BY SIMULATED MICROGRAVITY COCULTURE OF SERTOLI CELLS AND IMORTALIZED NT2 CELLS

Sertoli cells also have been utilized to facilitate the transplantation of dopaminergic cells into the brain as a treatment protocol for Parkinson's since Sertoli cells appear to provide localized immunoprotection at the graft site and to stimulate nerve cell viability (Sanberg, P.R., C.V. Borlongan, A.I. Othberg, S. Saporta, T.B. Freeman and D.F. Cameron. Testis-derived Sertoli cells have a trophic effect on dopamine neurons and alleviate hemiparkinsonism in rats. Mature medicine, 3(10):1129-1132.). To enhance this transplantation treatment protocol, as was utilized in the diabetes transplantation (see Example 1), Sertoli cells and the immortalized NT2 cell line were cocultured in simulated MICROGRAVITY using the NASA high aspect rotation velocity (HARV) bioreactor. Sertoli cells were isolated from peripuberital rats and placed immediately in HARVs along with the NT2 cells. Maintenance medium was DMEM/F12 supplemented with ITS+ and retinal and ± 1% Matrigel (MG). Cocultures were incubated at 37°C for one or two weeks in maintenance medium which was replaced when needed or every 48 hours. As with Sertoli cells and islets, cells organized to form Sertoli-neuron-aggregated cells (SNACs) (Figure 6).

At the time of SNACs collection, cell viability was determined by trypan blue exclusion. SNACs were processed for morphological analysis with 3% gluteraldehyde and processed into Epon/Araldite or fixed with 4% paraformaldehyde and processed into OCT for cryosectioning. Cryosections were immunostained for FasL (Sertoli cell marker), NuMa (NT2 cell marker) and tyrosine hydroxylase (TH-enzyme marker for dopamine synthesis).

Following the incubation period, cell viability was high (>90%) and there was segregation of Sertoli cells (peripherally distributed) and NT2 cells (centrally distributed) when incubated with MG. Positive FasL immunostaining was localized peripherally consistent with Sertoli cell distribution whereas NuMa localization was consistent with the distribution of

NT2 cells. Some centrally located cells showed positive immunostaining for TH. It appeared that with MG, the Sertoli biochamber tissue construct was achieved with these two cell types, as described for the SICA (see Example 1). It is therefore concluded that the HARV coculture of Sertoli cells, and NT2 neurons with MG, resulted in the formation of NT2-filled Sertoli biochambers comprised of FasL positive Sertoli cells forming the biochamber wall and NuMa positive NT2 cells residing within the biochamber. The expression of TH suggests that some of the NT2 cells had differentiated into the dopaminergic phenotype indicating the use of these SNACs transplantation protocols for the treatment of experimental Parkinson's disease.

EXAMPLE 3

Isolated Sertoli cells from peripubertal rats and pancreatic islets from neonatal pigs were co-cultured by conventional culture technology in the same medium described for the HARV simulated microgravity coculture. Sertoli cells were pre-plated 48 hours on plastic or Matrigel substrates. Pre-treated isolated pig islets were added to the Sertoli cell-enriched monoculture 24 hours later. This Sertoli-Islet co-culture was incubated at 37°C and within 24 hr. islets had attached to and integrated into the underlying Sertoli cells. Within another 48-72hrs, Sertoli cells reorganized into spherical or chord-like aggregates. This process was enhanced for those co-cultures in which Sertoli cells had been plated on the Matrigel. Islets appeared to retain their structural integrity better in the non-Matrigel co-cultures (Figure 4) than in the cocultures not having a Matrigel substrate (Figure 5). Tissue constructs of Sertoli cells and pancreatic islet cells can be created in conventional coculture in a similar manner as that observed in simulated microgravity coculture.

Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

REFERENCES

1. Kaufman, et al. (1990).
2. Evans, et al. (1990).
3. Horaguchi, et al. (1981).
4. Kneteman, et al. (1990).
5. Ricordi, et al. (1990).
6. Thompson et al. (1990).
7. Calafiore, et al. (1990).
8. Gray, et al. (1984).
9. Scharp, et al. (1987).
10. Alderson, et al. (1987).
11. Ricordi, et al. (1989).
12. Scharp, D.W. (1988).
13. Kneteman, et al. (1986).
14. Warmock, et al. (1988).
15. Warnock, et al. (1989).
16. Kuhn, et al. (1985).
17. Naji, et al. (1981).
18. Weringer, et al. (1985).
19. Prowse, et al. (1986).
20. Selawry (1985).
21. Selawry et al. (1987).
22. Selawry, et al. (1986).
23. Whitmore, et al. (1978).
24. Head, et al. (1983).
25. Head, et al. (1983).

26. Hedger, M.P. (1989).
27. Barker et al. (1968).
28. Selawry, et al. (1989).
29. Martin, D.C. (1982).
30. Cameron, et al. (1990).
31. Fawcett, et al. (1973).
32. Born, et al. (1982).
33. Born, et al. (1981).
34. Williams, et al. (1978).
35. Selawry, et al. (1993).
36. Bardin, et al. (1988).
37. Griswold, M.D. (1993).
38. Selawry, et al. (1991).
39. DeCesarts, et al. (1992).
40. Cantrell, et al. (1984).
41. Leapman, et al. (1981).
42. Hess, A.D. (1985).
43. Green, et al. (1978).
44. Homan, et al. (1980).
45. Bellgrau, et al. (1995).
46. Lau, et al. (1996).
47. Wickelgren, L. (1996).
48. Selawry, et al. (1996).
49. Sanberg, et al. (1995).
50. Sanberg, et al. (1966).
51. Sanberg, et al. (1996).
52. Borlongan, et al. (1996).
53. Cameron, et al. (1996).
54. Gondos, et al. (1993).

55. Skinner, et al. (1993).
56. Edgington, S.M. (1992).
57. Goodwin, et al. (1993).
58. Goodwin, et al. (1993).
59. Goodwin, et al. (1992).
60. Becker et al. (1993).
67. Cameron et al. (1991).
68. Cameron, et al. (1993).
69. London, et al. (199).
70. Cameron et al. (1990).
71. Schwarz, et al. (1992).
72. Goodwin, et al. (1996).
73. Suda, et al. (1993).
74. Towbin, et al. (1979).
75. Prewett, et al. (1993).
76. Goodwin, et al. 91993).
77. Goodwin, et al. (1993).

CLAIMS

What is claimed is:

1. A biological chamber system comprising a biochamber and center lumen, said biochamber being defined by outer walls of an engineered Sertoli tissue construct.

2. The system according to claim 1, wherein said engineered Sertoli tissue construct form said center lumen surrounding a population of cells which are different than said engineered Sertoli tissue construct .

3. The system according to claim 2, wherein said center lumen contains pancreatic islet cells.

4. The system according to claim 2, wherein said center lumen contains neuronal cells.

5. The system according to claim 4, wherein said neuronal cells are NT2 neurons.

6. The system according to claim 1, wherein said outer walls are formed from a plurality of engineered Sertoli cells to form the tissue construct.

7. The system according to claim 6, wherein said outer walls comprise a immunoprotective system.

8. The system according to claim 1, wherein said biological chamber system is used for transplantation.

9. A transplantation facilitator comprising a biochamber.

10. The transplantation facilitator according to claim 9, wherein said biochamber is defined as having outer walls formed of engineered Sertoli tissue construct.

11. The transplantation facilitator according to claim 10, wherein said biochamber is further defined as having a center lumen surrounding therapeutic cells.

12. The transplantation facilitator according to claim 11, wherein said therapeutic cells are neuronal cells.

13. A method of making biochambers comprising the steps of:

co-culturing facilitator cells and therapeutic cells about the therapeutic cells to form a chamber thereabout; and

re-engineering the facilitator cells to form a tissue construct.

14. The method according to claim 13, further including the step of segregating the facilitator cells away from the therapeutic cells.

15. The method according to claim 14, wherein said segregating step further includes the step of inducing the epithelization and polarization of the facilitator cells.

16. The method according to claim 17, wherein said inducing step further includes adding a compound for inducing epithelization and polarization.

17. A method of transplanting cells comprising the steps of:
forming a biochamber,
incorporating therapeutic cells into said biochamber; and
transplanting the biochamber containing the transplant cells into a host

18. The method according to claim 17, wherein said incorporating step further includes co-culturing the therapeutic cells with cells which form the biochamber simultaneously.

19. A biochamber comprising an outer wall of facilitator cells and an inner lumen of therapeutic cells.

20. A biochamber comprising an outer wall of protective cells and an inner wall of secreting cells, said outer wall allowing for release from said biochamber of the secretions from said secreting cells.

21. A transplantation vessel comprising a housing made of one type of cell including an inner cavity and a center lumen surrounding a population of cells different from said housing.

22. The transplantation vessel according to claim 21, wherein said housing consists of engineered Sertoli tissue construct.

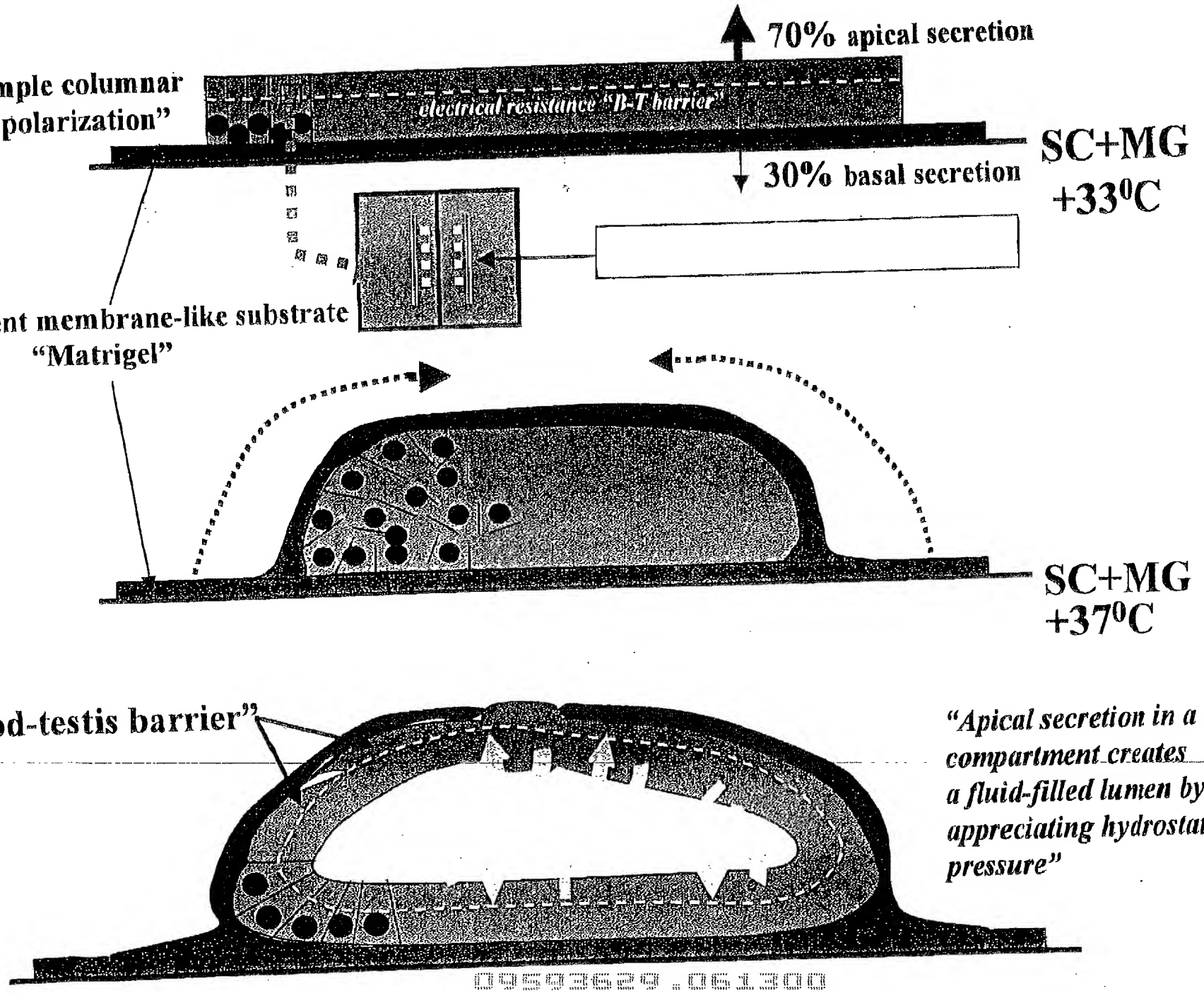
23. The transplantation vessel according to claim 21, wherein said cell population consists of at least one therapeutic cell.

24. The transplantation vessel according to claim 23, wherein said therapeutic cells are selected from the group consisting essentially or neuronal cells, NT2 cells, pancreatic islet cells, dopaminergic cells, and bovine chromaffin cells.

SERTOLI CELLS AS BIOCHAMBERS

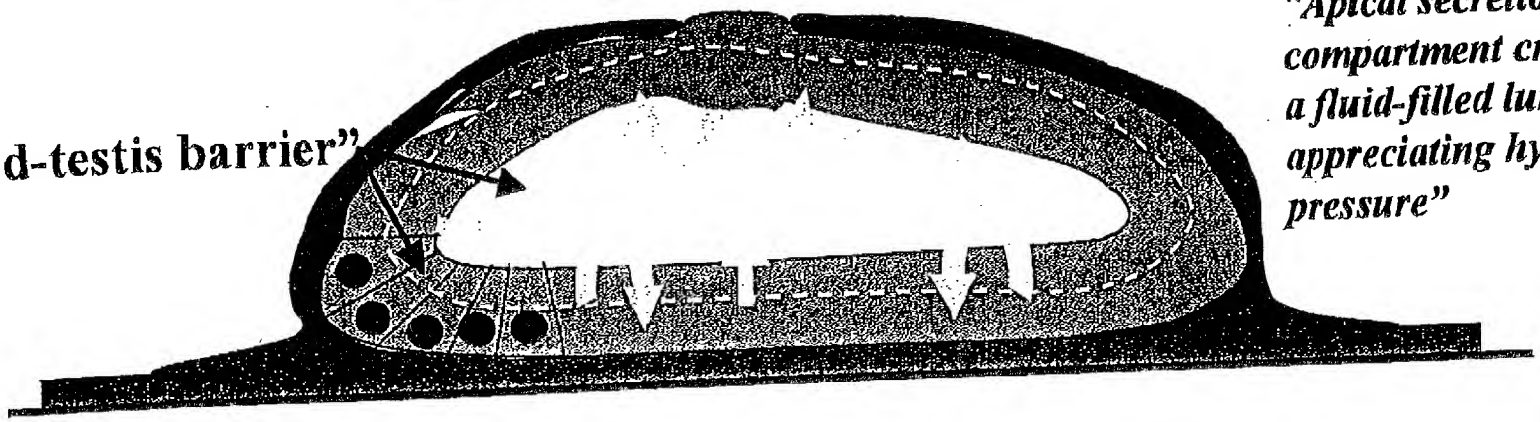
ABSTRACT OF THE DISCLOSURE

According to the present invention, there is provided a biological chamber system having a biochamber defined by outer walls of Sertoli cells. Also provided is a transplantation facilitator including a biochamber. A method of making biochambers by co-culturing facilitator cells and therapeutic cells and then aggregating the facilitator cells is also provided. Also provided is a method of transplanting cells by incorporating transplant cells into a biochamber and transplanting the biochamber containing the transplant cells.



"Apical secretion in a clos compartment creates a fluid-filled lumen by appreciating hydrostatic pressure"

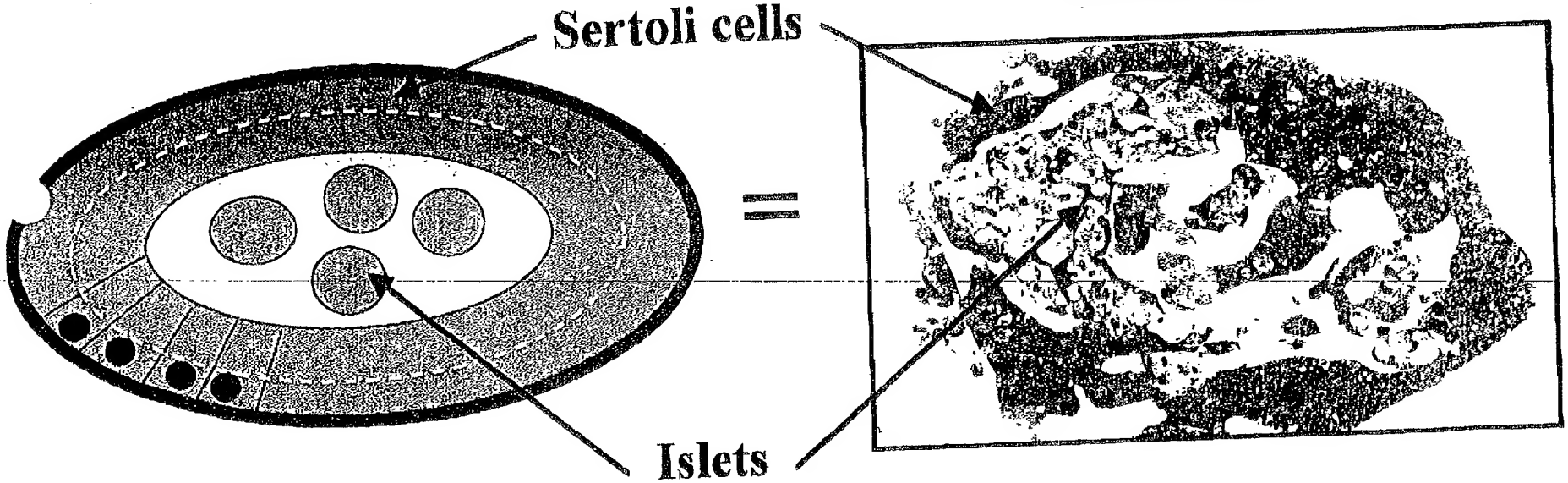
Conventional Culture

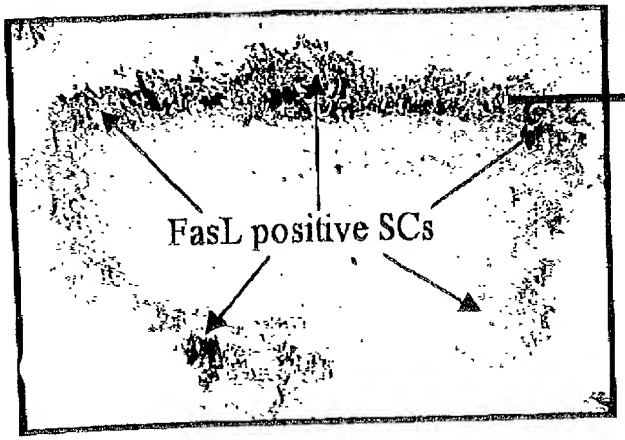


"Apical secretion in a closed compartment creates a fluid-filled lumen by appreciating hydrostatic pressure"

Microgravity Coculture

"Microgravity coculture results in th integration of therapeutic cells into Sertoli cell biochambers "





Activated T-lymphocytes with upregulated Fas receptors

FasL-Fas induced apoptosis of lymphocytes

FasL

Positive FasL immonstaining identifies Sertoli cells and suggests a mechanism by which they may effect immune suppression at the graft site.

Sertoli Biochamber

The expression of FasL by Sertoli cells induces apoptosis of the invading immune cells by binding to the upregulated Fas receptors on these activated T-lymphoctys. This results in the attrition of these immune cells at the graft site thereby downregulating the immue respones-this by an already well-defined mechanism occuring naturally in the mammalian system.

Addition to Disclosure "Sertoli Biochambers"
Cameron, Don F. et al.

Isolated Sertoli cells from peripubertal rats and pancreatic islets from neonatal pigs were cocultured by conventional culture technology in the same medium described for the HARV simulated microgravity coculture. Sertoli cells were pre-plated 48 hours on plastic or Matrigel substrates. Pre-treated isolated pig islets were added to the Sertoli cell-enriched monoculture 24 hours later. This Sertoli-Islet coculture was incubated at 37°C and by 24 hr islets attached to and integrated into the underlying Sertoli cells. Within antother 48-72hrs, Sertoli cells reorganized into spherical or chord-like aggregates. This process was enhanced for those cocultures in which Sertoli cells had been plated on the Matrigel. Islets appeared to retain their structural integrity better in the non-Matrigel cocultures (Fig 1) than in the cocultures not having a Matrigel substrate (Fig 2). Tissue constructs of Sertoli cells and pancreatic islet cells can be created in conventional coculture in a similar manner as that observed in simulated microgravity coculture.

Fig 1 Sertoli cell (SCs) and islets (arrows) in a Sertoli-islet tissue construct created in conventional coculture. B-cells are immunostained for insulin.

Fig 4

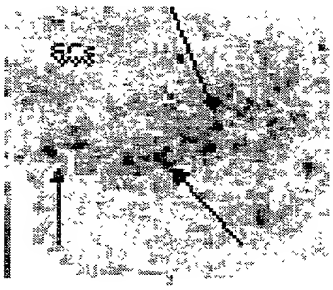


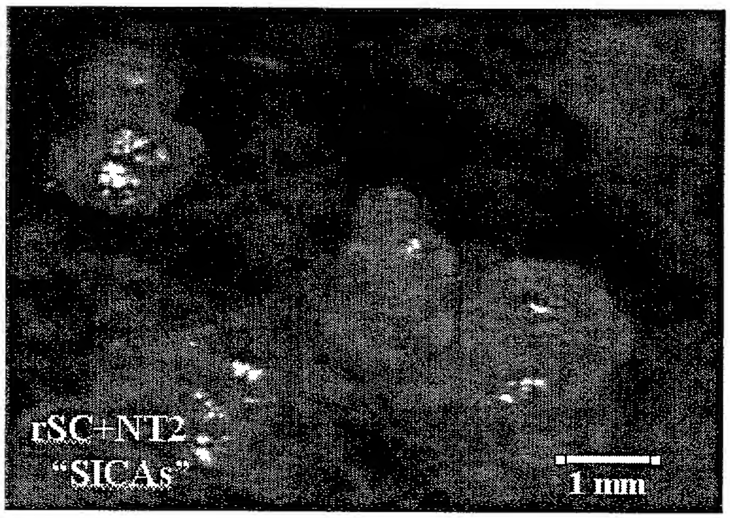
Fig 2. Sertoli cells (SCs) and B-cells (arrows) in a Sertoli-islet tissue construct created in conventional coculture, B-cells are immunostained for insulin.

Fig 5

1. Sanberg, P.R., C.V. Borlongan, A.I. Othberg, S. Saporta, T.B. Freeman and D.F. Cameron. Testis-derived Sertoli cells have a trophic effect on dopamine neurons and alleviate hemiparkinsonism in rats. Mature medicine, 3(10):1129-1132.

Figure 1.

1 Week HARV Coculture - rSCs + NT2



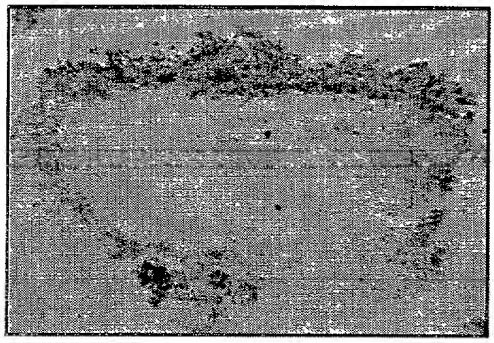
Sertoli-Neuron-Aggregatee-Cells (SNACs) fom *in vitro* following coculture of rat Sertoli cells and NT2 neuros in simulated micrograyity utilizing the High Aspect Rotation Velocity (HARV) bioreactor.

Fig 6

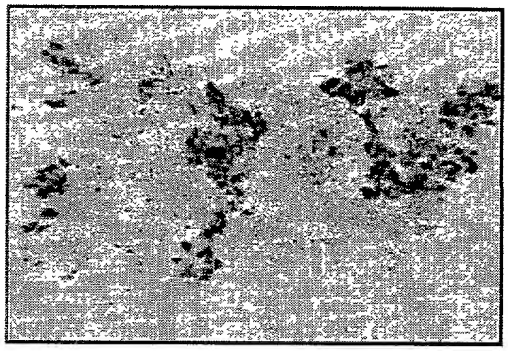
Figure 2.

1 Week HARV Coculture - rSCs + hNT2 (neurons)

NT2 + SC
FasL



NT2 + SC
hNuMu



Immucytochemical staining of mouse FasL and human nuclear matrix proteins in rSertoli-hNeuron Aggregated Cells (SNACs) following HARV incubated cocultures.

Fig 7

Docket No.
0152.00372

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

SERTOLI CELLS AS BIOCHAMBERS

the specification of which

(check one)

- ☒ is attached hereto.
- ☐ was filed on _____ as United States Application No. or PCT International Application Number _____ and was amended on _____ (if applicable)

☐ I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

☐ I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

Kenneth I. Kohn, Reg. No. 30,955
Amy E. Rinaldo, Reg. No. 45,791

Send Correspondence to: **Amy E. Rinaldo**
KOHN & ASSOCIATES
30500 Northwestern Highway, Suite 410
Farmington Hills, Michigan 48334

Direct Telephone Calls to: *(name and telephone number)*
Amy E. Rinaldo (248) 539-5050

Full name of sole or first inventor **Don F. Cameron**
Sole or first inventor's signature Date
Residence **18206 Clear Lake Drive, Lutz,Florida 33549**
Citizenship **U.S.**
Post Office Address **Same as above.**

Full name of second inventor, if any **Paul R. Sanberg**
Second inventor's signature Date
Residence **11751 Pilot Country Drive, Spring Hills, Florida 34610**
Citizenship **U.S.**
Post Office Address **Same as above.**

Full name of third inventor, if any
Samuel Saporta

Third inventor's signature

Date

Residence
14026 Shady Shores Drive, Tampa, Florida 33613

Citizenship
U.S.

Post Office Address
Same as above.

Full name of fourth inventor, if any
Joel J. Hushen

Fourth inventor's signature

Date

Residence
16914 Harrierridge Place, Lithia, Florida 33547

Citizenship
U.S.

Post Office Address
Same as above.

Full name of fifth inventor, if any

Fifth inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of sixth inventor, if any

Sixth inventor's signature

Date

Residence

Citizenship

Post Office Address